

AID Produces DNA Double-Strand Breaks in Non-*Ig* Genes and Mature B Cell Lymphomas with Reciprocal Chromosome Translocations

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SUMMARY

Cancer-initiating translocations such as those associated with lymphomas require the formation of paired DNA double-strand breaks (DSBs). Activation-induced cytidine deaminase (AID) produces widespread somatic mutation in mature B cells; however, the extent of “off-target” DSB formation and its role in translocation-associated malignancy is unknown. Here, we show that deregulated expression of AID causes widespread genome instability, which alone is insufficient to induce B cell lymphoma; transformation requires concomitant loss of the tumor suppressor p53. Mature B cell lymphomas arising as a result of deregulated AID expression are phenotypically diverse and harbor clonal reciprocal translocations involving a group of *Immunoglobulin (Ig)* and non-*Ig* genes that are direct targets of AID. This group includes *miR-142*, a previously unknown micro-RNA target that is translocated in human B cell malignancy. We conclude that AID produces DSBs throughout the genome, which can lead to lymphoma-associated chromosome translocations in mature B cells.

INTRODUCTION

Chromosome translocations are characteristic features of several different forms of B cell cancers in mice and humans, including lymphomas and myelomas (Kuppers, 2005; Kuppers and Dalla-Favera, 2001; Potter, 2003). These karyotypic abnormalities are etiologically important in malignancy because they either deregulate oncogene expression or create novel oncogenes by bringing together disparate transcription units (Rab-

bitts, 2009). Chromosome translocations are believed to be particularly frequent in mature B lymphocytes because, in addition to V(D)J rearrangements early in development, these cells undergo two forms of programmed DNA damage in their immunoglobulin (*Ig*) loci during immune responses: somatic hypermutation and class switch recombination (Peled et al., 2008; Stavnezer et al., 2008; Teng and Papavasiliou, 2007).

Somatic hypermutation introduces nontemplated point mutations in antibody variable genes, and class switch recombination replaces one constant region for another by a deletional recombination reaction (Peled et al., 2008; Stavnezer et al., 2008; Teng and Papavasiliou, 2007). Although the two reactions are mechanistically distinct, both are initiated by a single enzyme, AID, which is believed to act by deaminating cytosine to produce U:G mismatches in target DNA (Di Noia and Neuberger, 2007; Honjo et al., 2002; Muramatsu et al., 2000). Diverse and overlapping DNA repair pathways process these lesions to produce somatic mutations, or DNA double-strand breaks (DSB), which are obligate intermediates in the class switch reaction (Peled et al., 2008). These same DSBs can also become substrates for chromosome translocations (Jankovic et al., 2007; Ramiro et al., 2006b, 2007). However, a single DSB at *Ig* alone is not sufficient for translocation (Robbiani et al., 2008). To account for the DSBs in non-*Ig* genes, it has been proposed that the enzymes that create the breaks in *Ig* loci, RAG1/2 and AID, can also cause damage in non-*Ig* genes (Lieber et al., 2008; Tsai et al., 2008). Consistent with this idea, we found that the oncogene *c-myc* is a target of AID, although the frequency of DSB formation at *c-myc* is significantly lower than at *IgH* (Robbiani et al., 2008).

Burkitt's lymphoma, diffuse large B cell lymphoma, and multiple myeloma represent different types of mature B cells that carry clonal translocations and may have expressed AID (Kuppers, 2005; Shaffer et al., 2002). A role for AID in inducing translocations in these cancers has been suggested by studies of activated B cells in vitro and plasmacytosis in IL-6 transgenic

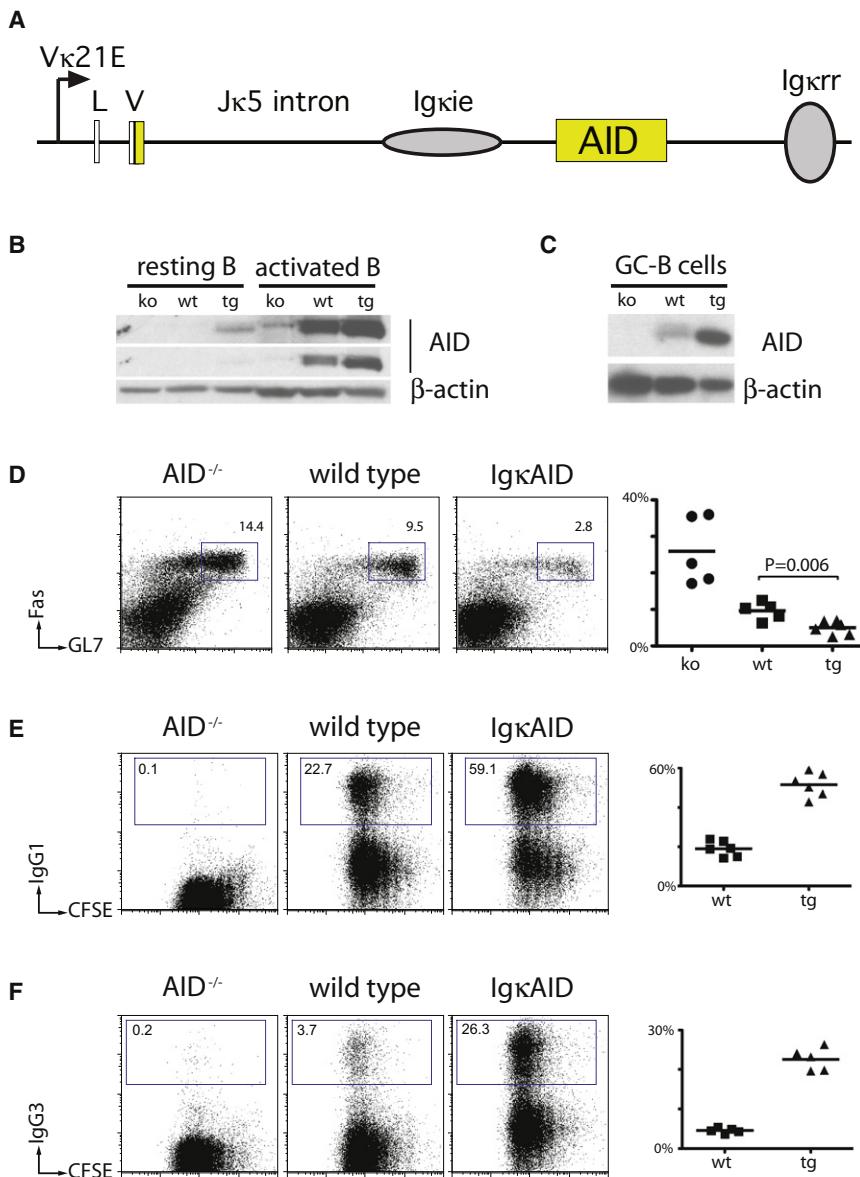


Figure 1. Increased Class Switching in *IgκAID* B Cells

(A) Schematic representation of the *IgκAID* transgene. AID cDNA (yellow) is embedded in *Igκ* regulatory elements (V_κ21E promoter, noncoding leader [L] and V gene exons, intronic sequence with enhancer [ie], and 3' regulatory region [rr]). (B and C) Western blot for AID protein in *IgκAID* B cells. (B) One million cells of the indicated genotypes were assayed in each lane. Top and middle are different exposures of the same blot. (C) 0.5 million sorted germinal center (GC) B cells were assayed in each lane.

(D) Flow cytometric analysis of Peyer's patch germinal center B cells from matched 6- to 10-week-old mice. Graph (right) shows a summary of two independent experiments with 2–3 mice each. Error bar indicates the mean value for each group (25.9% ko, 9.6% WT, 5.0% tg). P value was calculated by unpaired t test.

(E and F) Flow cytometric analysis for class switching to IgG1 and IgG3 and cell division. Graph (right) shows a summary of two independent experiments with 2–3 mice each. Error bar indicates the mean value for each group (IgG1: 18.9% WT, 51.6% tg; IgG3: 3.8% WT, 22.6% tg).

issue of whether AID can induce a sufficiently varied genomic damage to account for *Ig* and non-*Ig* gene translocation-associated B lymphocyte malignancy in vivo remains to be determined.

RESULTS

Increased Switching, Mutation, and *c-myc/IgH* Translocation in AID Transgenic Mice

To determine whether deregulated AID expression can destabilize the genome and induce B cell malignancy in vivo, we produced transgenic mice that express AID under the control of *Igκ* regulatory

elements (*IgκAID*) (Figure 1A). AID is expressed at low levels in resting B cells in *IgκAID* mice and increases in activated and germinal center B cells (Figures 1B and 1C and Figure S1 available online). Nevertheless, with the exception of the expected decrease in germinal center cells (Figure 1D; Dorsett et al., 2008; Muramatsu et al., 2000), B cell development and distribution is normal in *IgκAID* mice, and serum Ig isotypes are not increased (Figures S2 and S3). Consistent with the increase in AID expression in stimulated B cells in vitro, *IgκAID* B cells switched at higher frequency to IgG1 and IgG3 (Figures 1E and 1F). Thus, although deregulated AID expression does not significantly alter B cell development in vivo, it does lead to increased levels of class switch recombination in vitro. This effect is consistent with the observation that AID concentrations are limiting for this reaction (Dorsett et al., 2008; McBride et al., 2006; Takizawa et al., 2008).

Curiously, however, deregulated AID does not cause malignancy or translocation-associated cancer in B cells (Muto et al., 2006; Okazaki et al., 2003; Shen et al., 2008). In addition, despite its obligate role in *c-myc/IgH* translocation, AID is not required for the development of plasmacytosis or plasmacytoma in IL-6 transgenic or pristane-treated mice, respectively (Kovalchuk et al., 2007; Ramiro et al., 2004). Finally, most human B cell lymphoma-associated translocations do not involve *c-myc*, and many do not involve *Ig* genes (Kuppers, 2005). Thus, the

elements (*IgκAID*) (Figure 1A). AID is expressed at low levels in resting B cells in *IgκAID* mice and increases in activated and germinal center B cells (Figures 1B and 1C and Figure S1 available online). Nevertheless, with the exception of the expected decrease in germinal center cells (Figure 1D; Dorsett et al., 2008; Muramatsu et al., 2000), B cell development and distribution is normal in *IgκAID* mice, and serum Ig isotypes are not increased (Figures S2 and S3). Consistent with the increase in AID expression in stimulated B cells in vitro, *IgκAID* B cells switched at higher frequency to IgG1 and IgG3 (Figures 1E and 1F). Thus, although deregulated AID expression does not significantly alter B cell development in vivo, it does lead to increased levels of class switch recombination in vitro. This effect is consistent with the observation that AID concentrations are limiting for this reaction (Dorsett et al., 2008; McBride et al., 2006; Takizawa et al., 2008).

To determine whether transgenic AID expression might alter its targeting, we examined the extent of somatic mutation in documented AID target (*IgV_H*, *S μ* , *c-myc*) and nontarget genes (*Taci*, *Whsc1*, *H2E α* , *A20*), all of which are expressed in stimulated B cells (Figure S4; Liu et al., 2008). *IgV_H* mutation was measured by combining the B1-8^{hi} *IgV_H* knockin allele (Casellas et al., 2001) with *Ig κ AID* (*Ig κ AID/B1-8^{hi}*) or *AID*^{-/-} controls (*AID*^{-/-}/B1-8^{hi}). Consistent with constitutive AID expression in naive B cells, resting *Ig κ AID/B1-8^{hi}* B cells showed high levels of mutation in *IgV_H* and *S μ* and only a modest increase upon in vitro activation with LPS and IL-4 (Figure 2A). *IgV_H* and *S μ* mutation was not significantly detectable in controls but was found at rates of 7.5×10^{-4} and 51×10^{-4} , respectively, in naive AID transgenic B cells. This rate of mutation is one order of magnitude lower than the rates normally found in germinal center B cells (Liu et al., 2008; McKean et al., 1984). In contrast, the increased levels of AID did not result in hypermutation in any of the nontarget genes tested (Figure 2A). Also consistent with a physiologic pattern of target selection, *c-myc*, which has a propensity toward error-free DNA repair (Liu et al., 2008), shows only a modest increase in mutation rate in activated *Ig κ AID* B cells (1.43×10^{-4} versus 0.64×10^{-4}). We conclude that deregulated expression of AID induces hypermutation in resting B cells at levels close to those normally found in germinal center cells; however, the specificity of target selection is maintained because there is no significant DNA damage in nontarget genes.

Even though *c-myc* is a bona fide target of AID, DSBs at *c-myc* and *c-myc/IgH* translocations are difficult to detect under physiological conditions (Robbiani et al., 2008). Despite constitutive AID expression in *Ig κ AID* B cells, we found no *c-myc/IgH* translocations in resting transgenic B cells (Figure 2B). In contrast, *Ig κ AID* B cells stimulated with LPS and IL-4 showed an increase in translocation frequency when compared to controls (Figure 2B). Therefore, transgenic B cell expression increases the frequency of *c-myc/IgH* translocations in activated B cells.

B Cell Lymphoma

Despite high levels of *c-myc/IgH* translocations in stimulated B cells expressing deregulated AID, B cell lymphomas did not develop in *Ig κ AID* or in three other previously reported strains of AID transgenic mice (Figure 3A; Muto et al., 2006; Okazaki et al., 2003; Shen et al., 2008). All eight of the *Ig κ AID* mice survived beyond 40 weeks (Figure 3A). One potential explanation for this unexpected result is that B cells are protected from AID-mediated DNA damage-induced transformation by gatekeepers such as p53, which trigger cell-cycle arrest or apoptosis (Bassing et al., 2003; Green and Kroemer, 2009; Kruse and Gu, 2009; Lowe et al., 2004; Ramiro et al., 2006a). Consistent with this idea, gatekeeper genes or genes that control them, such as *Bcl6* (Phan and Dalla-Favera, 2004), are frequently deleted or mutated in mature human B cell lymphomas (Kuppers, 2005).

To determine whether p53 is protective against AID-induced B cell lymphomas in vivo, we produced *Ig κ AID/p53*^{-/-} mice. In contrast to *Ig κ AID* mice, all 30 of the *Ig κ AID/p53*^{-/-} mice died within 28 weeks, and they succumbed more rapidly to malignancy than did *p53*^{-/-} controls ($p < 0.0001$; Figures 3A and 3B). In addition, tumors that developed in *Ig κ AID/p53*^{-/-} mice

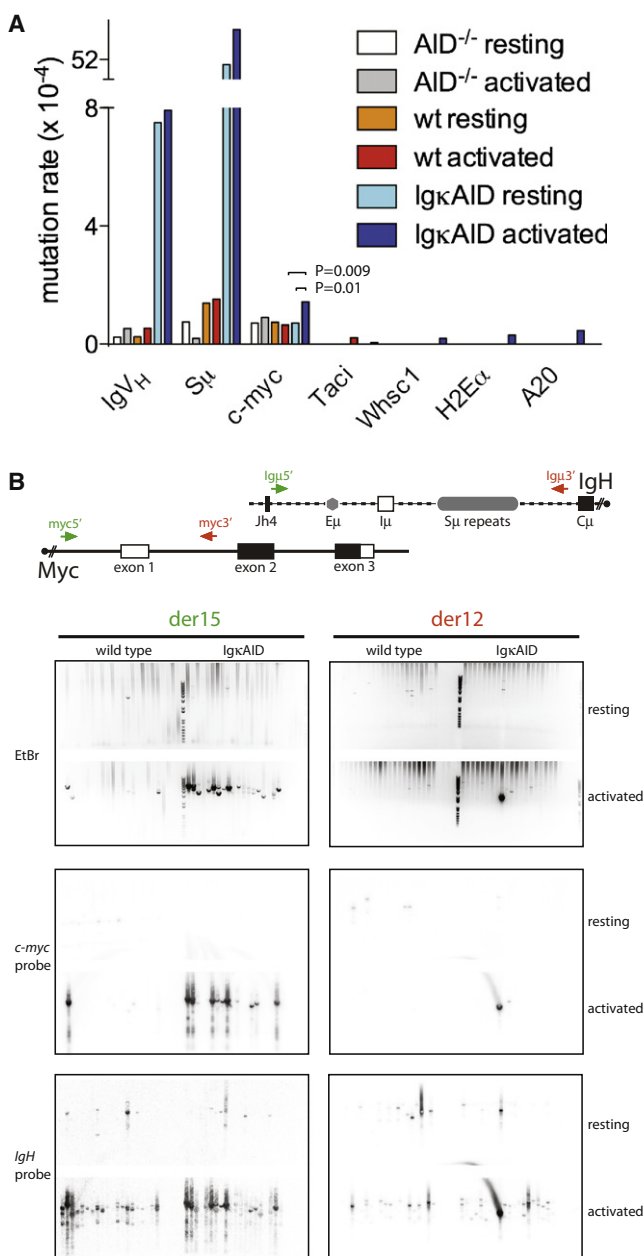


Figure 2. Somatic Mutations and *c-myc/IgH* Translocations

(A) Frequency of somatic mutations in indicated loci of resting and LPS- and IL-4-stimulated B cells.

(B) *C-myc/IgH* translocations in resting B cells or B cells activated with LPS and IL-4 for 4 days. (Top) Schematic representation of the *Myc* and *IgH* alleles with the PCR primers for detecting der15 and der12 *c-myc/IgH* translocations. (Bottom) Ethidium bromide (EtBr)-stained gel with PCR bands indicative of translocations was blotted and probed for *c-myc* and *IgH*, as indicated, to verify translocations. Translocation frequency: wild-type 0.4×10^{-6} (der15) and 0.1×10^{-6} (der12); *Ig κ AID* 4.6×10^{-6} (der15) and 1.3×10^{-6} (der12). One of two independent experiments is shown.

differed from those in the *p53*^{-/-} controls: the former were predominantly mature B cell lymphomas, whereas the *p53*^{-/-} controls developed thymic lymphomas and sarcomas (Figure 3B;

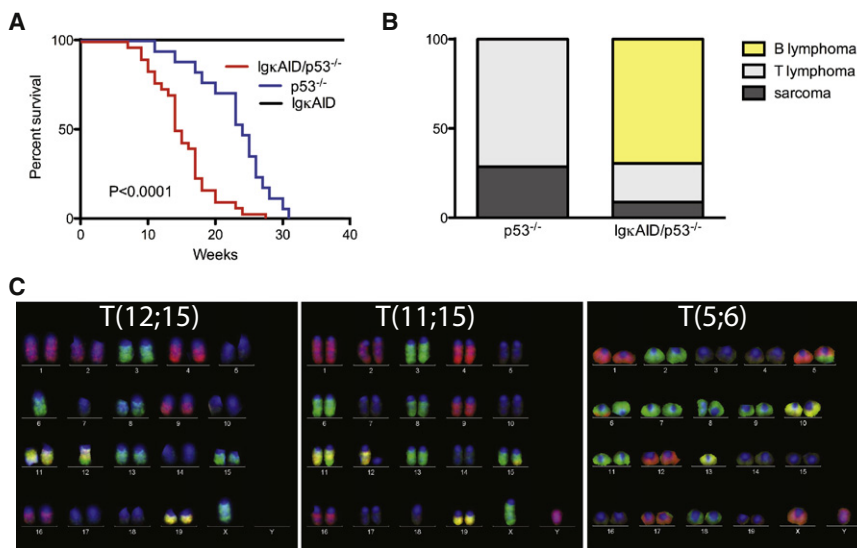


Figure 3. *IgkAID/p53^{-/-}* Mice Succumb to B Cell Lymphomas with Reciprocal Translocations

(A) Survival curve for $n = 8$ *IgkAID*, $n = 17$ *p53^{-/-}*, and $n = 30$ *IgkAID/p53^{-/-}* mice. The P value refers to the difference between *IgkAID/p53^{-/-}* and *p53^{-/-}* (log rank test). The median survival for *IgkAID/p53^{-/-}* is 14.5 weeks; for *p53^{-/-}*, it is 24 weeks.

(B) Phenotype of tumors at necropsy ($n = 7$, *p53^{-/-}*; $n = 23$, *IgkAID/p53^{-/-}*).

(C) Representative M-FISH images of reciprocal translocations found in B cell lymphomas of *IgkAID/p53^{-/-}* mice.

Tumor-Associated Translocations Induced by AID In Vivo

Nine of the eleven *IgkAID/p53^{-/-}* B cell lymphomas analyzed by M-FISH showed a clonal T(11;15) translocation; the other

Jacks et al., 1994). The mature B cell lymphomas in *IgkAID/p53^{-/-}* mice represent a broad spectrum of the different stages of B cell development—from naive B cells that express high levels of IgM and little or no IgD to plasmacytomas that express CD138 and no surface Ig (Table 1 and Figure S5). We conclude that deregulated AID expression in a *p53*-deficient background leads to the emergence of a broad spectrum of mature B cell lymphomas.

To determine whether *IgkAID/p53^{-/-}* lymphomas were associated with chromosome translocations, we performed multi-color-FISH (M-FISH) analysis on tumor metaphase spreads. We found that 11 out of the 12 *IgkAID/p53^{-/-}* B cell lymphomas tested contained a clonal reciprocal translocation. In all cases, additional nonclonal chromosome aberrations were present (Figure 2C and Table 1). In contrast, clonal reciprocal translocations were not observed in the *p53^{-/-}* control T cell lymphomas (data not shown and Liao et al., 1998). Thus, in the absence of *p53*, deregulated AID can produce B cell lymphomas that harbor clonal reciprocal chromosome translocations similar to those that are characteristic of human B cell lymphomas.

two exhibited a T(12;15) or T(5;6) translocation, with a variety of additional clonal and nonclonal chromosomal abnormalities (Table 1). The tumor with T(12;15) was a typical mouse plasmacytoma that expressed CD19 and high levels of CD138, but not surface Ig (Table 1 and Figure S5). PCR analysis was used to determine that the translocation involved *c-myc* and *IgH*, and sequence analysis revealed that the breakpoint on *c-myc* was in the promoter region and that the breakpoint at the *IgH* locus was in the IgH switch μ region (Table S1). Such reciprocal translocation is similar to those observed in human Burkitt's lymphomas and mouse plasmacytomas and are consistent with *IgH* and *c-myc* being targets of AID activity (Robbiani et al., 2008).

To identify the translocation breakpoints in tumors containing T(11;15) and T(5;6), we performed high-resolution array painting. Because the translocations in *IgkAID/p53^{-/-}* B cell lymphomas appeared to be balanced as determined by M-FISH (Figure 3C), we sorted derivative chromosomes from the tumor samples to compare with normal reference DNA (Gribble et al., 2007). Translocation breakpoints and copy number changes were revealed as discontinuities (high-low or low-high ratio) in the hybridization

Table 1. Karyotype and Phenotype of B Lymphomas in *IgkAID/p53^{-/-}*

Tumor ID	Clonal Translocation	Additional Changes in At Least One Metaphase	Tumor Phenotype
#1461	T(11;15)	Dic(13;13)	CD19 ⁺ IgM ⁺ IgD ⁻
#1535	T(11;15)	-12, +13, -19	CD19 ⁺ IgM ^{dim} IgD ^{+/-}
#1536	T(5;6)	-13, T(6;19)	CD19 ⁺ IgM ⁺ IgD ^{dim}
#1550	T(11;15)	-13, -16, -17, -18, T(16;17), T(16;Y)	CD19 ⁺ IgM ⁺ IgD ^{dim/+} CD138 ^{+/-}
#1561	T(11;15)	+3, -12, -Y, T(12;16), T(12;17)	CD19 ⁺ IgM ⁺ IgD ⁻ CD138 ^{dim}
#1583	T(11;15)	-12, -13, -14	CD19 ⁺ IgM ^{+/++} IgD ^{+/-} CD138 ^{dim}
#1584	T(11;15)	-12, -18	CD19 ⁺ IgM ^{+/++} IgD ^{+/-} CD138 ^{dim}
#1589	none	-5, -14, -18, -X, T(3;4), T(3;11), T(3;19)	CD19 ⁺ IgM ⁻ IgD ⁻ Igκ ⁻ CD138 ^{+/-}
#1592	T(11;15)	-7, +16	CD19 ⁺ IgM ⁺ IgD ^{+/-} CD138 ^{+/-}
#1593	T(12;15)	-7	CD138 ⁺⁺ CD19 ⁺ IgM ⁻ IgD ⁻ Igκ ⁻
#1594	T(11;15) T(12;17)	T(2;11), T(9;15), T(11;16), T(13;5)	CD138 ⁺⁺ CD19 ⁺ IgM ⁻ IgD ⁻ Igκ ⁻
#1595	T(11;15)	-12, -13, -19, T(13;16)	CD19 ⁺ IgM ⁺ IgD ⁻ CD138 ^{+/-}

ratio of sorted derivative chromosomes compared to unsorted reference DNA. We found that the breakpoints in the T(11;15) translocation were in the region of *micro-RNA 142* (*miR-142*) and *c-myc* and in T(5;6) at *Anxa4* and *Wdfy3* (Figures 4A, 4B, and S6). The apposition of *miR-142* on chr11 with *c-myc* on chr15 was confirmed by Southern blotting (Figure 4C). The high-resolution mapping by array painting allowed for the design of PCR primers to characterize fourteen T(11;15) translocations molecularly. The *miR-142* breakpoints were clustered in 1.8 kbps surrounding the micro-RNA, whereas the breakpoints at *c-myc* were in a 1.5 kbps region centered in intron 1 (Figure 4D). The resulting *miR-142/c-myc* fusion genes on der11 chromosome contained the *miR-142* promoter and the coding region of *c-myc*. These genes were associated with the production of fusion transcripts between *miR-142* and *c-myc* (der11), leading to the overexpression of *c-myc* at levels similar to those found in the S107 plasmacytoma, which carries a *c-myc/IgH* translocation (Figures 4E and 4F). Importantly, a closely related clonal translocation between *miR-142* and *c-myc* has been reported in human B cell leukemia (Gauwerky et al., 1989). We conclude that the T(11;15) translocation found in the vast majority of *IgκAID/p53*^{-/-} B cell cancers results in deregulated expression of *c-myc* by juxtaposition to regulatory elements from *miR-142*, one of the most highly expressed micro-RNAs in hematopoietic cells (Chen et al., 2004; Landgraf et al., 2007). Based on the B cell developmental stages, as well as karyotype and high-resolution analysis of the T(12;15) and T(11;15) translocations, it appears that the *IgκAID/p53*^{-/-} mouse provides a relevant model for studying the development of human B cell lymphoma in vivo.

AID might induce lymphoma-associated translocations by producing direct genomic damage or indirectly by compromising the genes that maintain genomic stability (Jankovic et al., 2007). The T(11;15) translocation junction sequences revealed either blunt end joining or small amounts of microhomology (Figure 4G and Table S1). In eight cases, we were able to characterize both derivative chromosomes. Surprisingly, six of these showed duplications in one or both of the translocated genes. In agreement with this observation, duplications were also present in the lymphomas with T(12;15) and T(5;6) translocation. In this setting, duplication could only be produced by filling in a break created by two staggered single-strand nicks, one on each DNA strand. This type of DSB is consistent with a staggered pair of lesions introduced by AID (Figures 4G and S7 and Table S2).

To determine whether any of the translocated genes are direct targets of AID, we obtained DNA sequences from primary resting and activated B cells (Figure 5A). We found a high rate of mutation in *miR-142* in resting (4.5×10^{-4}) and activated (7.8×10^{-4}) *IgκAID* B cells ($p = 0.0004$ and $p = 0.0007$, respectively; Figures 5A and S8), but not in *Anxa4* or *Wdfy3*. Hypermutation indicative of AID activity at *miR-142* was also detected in the region surrounding the translocation breakpoints as well as, more significantly, in the nontranslocated allele of T(11;15) lymphomas (Figures 4G and S9 and Table S1). Finally, we confirmed the in vitro results by analyzing *c-myc* and *miR-142* mutations in germinal center B cells. Both *c-myc* and *miR-142* were hypermutated in *IgκAID* germinal center B cells at levels similar to those

found in activated B cells in vitro (Figure 5B). In addition, both *c-myc* and *miR-142* mutations were enhanced in the absence of UNG in cells with endogenous levels of AID (Figure 5B). Therefore, *miR-142* resembles *c-myc* in that AID-induced lesions have a propensity toward error-free repair, which is UNG dependent (Figure 5B; Liu et al., 2008). Thus, the majority of the B cell lymphomas induced by deregulated AID expression involves direct AID target genes (a total of 15 T(12;15) and T(11;15)), whereas a minority (1 T(5;6)) may result indirectly from other genome-destabilizing mutations.

Genomic DNA Damage

To examine the spectrum of genomic DNA damage induced by AID deregulation in primary nontransformed B cells, we measured chromosome translocations by M-FISH (Figures 6A and 6B). We found that 4.4% of all *IgκAID/p53*^{-/-} B cells showed translocations in LPS-, IL-4-, and RP105-stimulated B cells ($n = 250$; Figure 6B). Metaphases containing translocations were rare in wild-type B cells (3 of 500 metaphases; Table S3; Callén et al., 2007; Ramiro et al., 2006a; Wang et al., 2009). Of interest, *IgκAID*, although tumor free, showed a similar frequency of translocation to *IgκAID/p53*^{-/-} (6%, $n = 500$ metaphases; Figure 6B and Table S3). Thus, p53 does not have an impact on the formation of translocations, and this factor must act downstream of translocation to prevent malignancy. Significantly, out of 500 metaphases from *AID*^{-/-} mice, none showed chromosome aberrations, indicating that the translocations in *IgκAID* mice are completely AID dependent (Figure 6B). Every chromosome (except Chr5, Chr14, Chr18, and ChrY) was found translocated in at least one metaphase from *IgκAID* B cells. The distribution of translocations was similar between *IgκAID/p53*^{-/-} and *IgκAID* mice (Figure 6C). Remarkably, some of the chromosomes were found at an extremely high frequency, including Chr12 (21 of 45 translocations), Chr16 (9 of 45 translocations), Chr 11, and Chr13 (both 7 of 45 translocations) (Figure 6C and Table S3). We conclude that, in addition to *c-myc/IgH* translocations, AID induces a large number of translocations in B cells and that loss of p53 does not increase the frequency of translocations.

Translocations are products of DNA breaks. To directly determine the frequency of DNA breaks, we examined DAPI-stained metaphase spreads that were hybridized with a telomere repeat-specific peptide nucleic acid probe marking chromosome ends (Figures 6A and 6B; Callén et al., 2007). The level of chromosome and chromatid breaks was similar in *IgκAID* (9%) and *IgκAID/p53*^{-/-} (8%) B cells, and this level of DNA damage was 8- to 9-fold higher than that found in *AID*^{-/-} mice (Figures 6A and 6B). Thus, deregulated AID expression produces widespread DNA damage that is independent of p53 and promotes chromosomal translocations in mature B cells.

DISCUSSION

Lymphoid malignancies account for 5% of all human cancers; the majority of these involve mature B cells at various stages of development. Chromosome translocations between *Ig* and non-*Ig* genes feature prominently in these cancers; however, they can also occur between two non-*Ig* genes. For example,

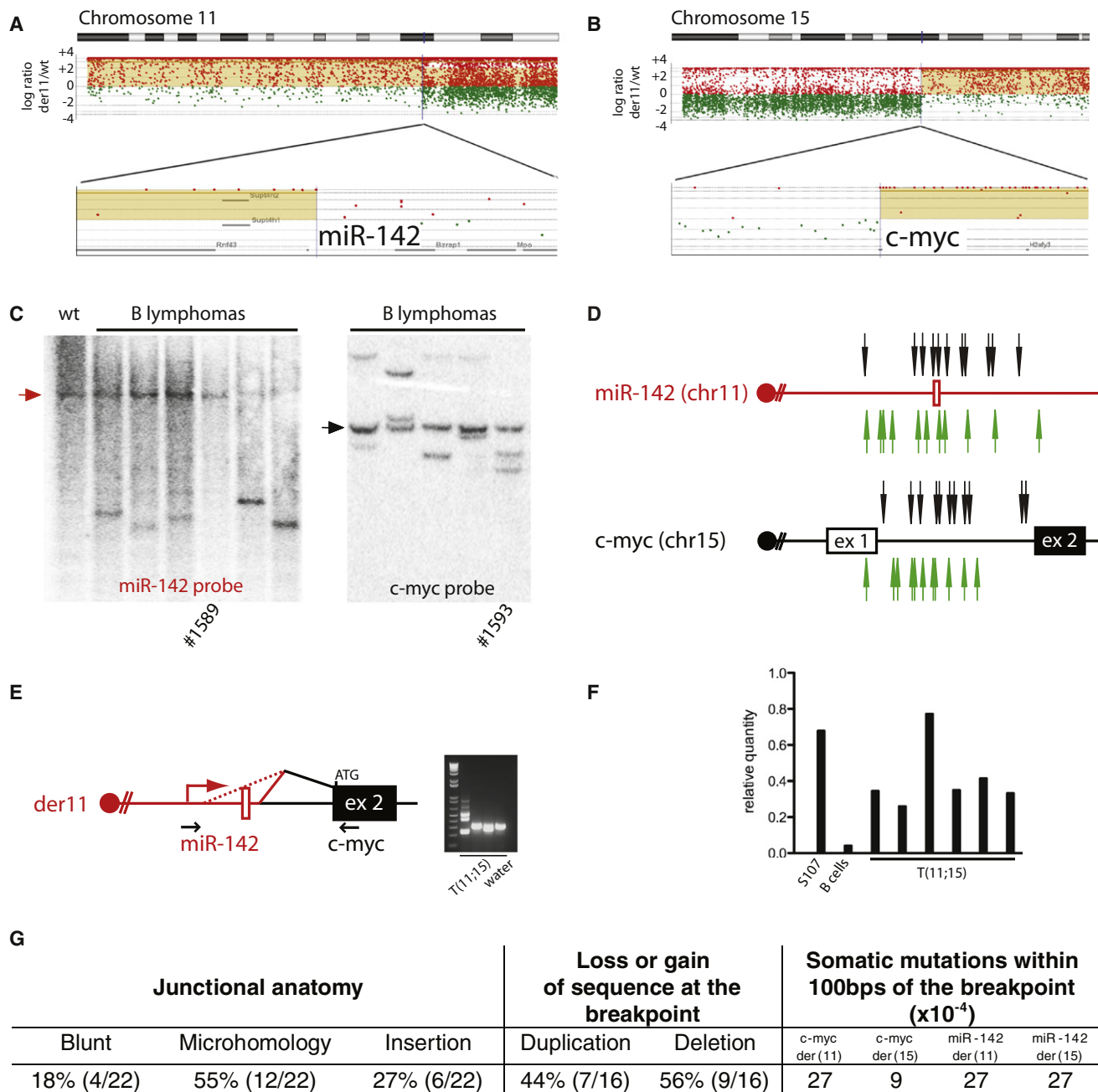


Figure 4. Identification of the T(11;15) Translocation Partners

(A and B) aCGH analysis of sorted chromosomes identifies the breakpoint in proximity of *miR-142* (Chr11) and *c-myc* (Chr15).

(C) Southern blotting with *c-myc* and *miR142* probes. Unless otherwise noted, tumors have T(11;15) by M-FISH. Arrows point to the unrearranged germline bands.

(D) Map of translocation breakpoints in T(11;15) B cell lymphomas from IgkAID/p53^{-/-} mice (n = 14; in addition to the nine T(11;15) identified by M-FISH, an additional five were identified by PCR). For some tumors, the breakpoint could be identified in one only derivative (see Table S1). Arrows point to translocation breakpoints for der11 (black) or der15 (green).

(E) RT-PCR for hybrid transcripts between *miR-142* and *c-myc*.

(F) qPCR for *c-myc* expression in T(11;15) tumors. S107 is a plasmacytoma cell line with *c-myc/IgH* translocation (positive control). Negative control is activated B cells.

(G) Features of the T(11;15) breakpoints.

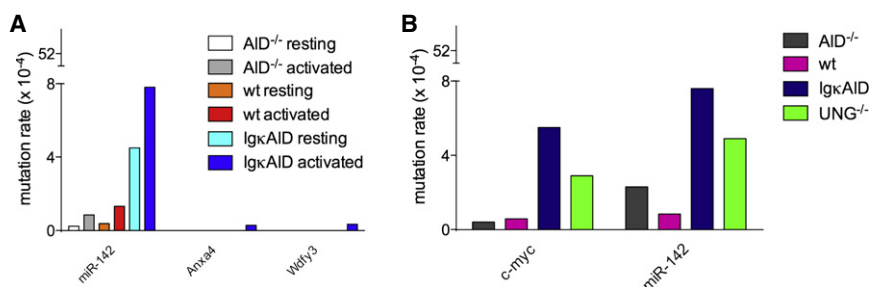


Figure 5. *miR-142* Is an AID Target

(A) Frequency of somatic mutations in *miR-142*, *Anxa4*, and *Wdfy3* in resting and LPS- and IL-4-stimulated B cells. (B) Frequency of somatic mutations in *miR-142* and *c-myc* in purified germinal center B cells from AID^{-/-}, wild-type, IgκAID, and UNG^{-/-} mice.

Burkitt's lymphomas typically contain *c-myc/IgH* translocations, whereas, in MALT lymphomas, the translocation commonly involves API2 and MALT (Inagaki, 2007). Multiple origins have been suggested for the paired DNA lesions that are obligate intermediates in lymphoid translocations, including the RAG1/2 V(D)J recombinase (Bassing et al., 2002; Callén et al., 2009; Dudley et al., 2005; Schatz, 2004; Schlissel et al., 2006; Tonegawa, 1983), AID (Ramiro et al., 2004, 2006a, 2007), reactive oxygen species, fragile sites, and combinations of the above (Callén et al., 2007; Greaves and Wiemels, 2003; Lieber et al., 2006, 2008; Tsai et al., 2008; Wang et al., 2009). However, none of these is directly demonstrated to produce the necessary initiating lesions on multiple chromosomes in lymphoid cells. Our experiments show that transgenic overexpression of AID in mice produces DSBs on most chromosomes and indicate that expression of this enzyme may be sufficient to account for many of the lesions that lead to mature B cell lymphoma.

AID is known to produce translocations between *c-myc* and *IgH* in B cells in vitro as well as in IL-6 transgenic plasmacytes and pristane-induced plasmacytomas (Dorsett et al., 2007; Ramiro et al., 2004, 2006a; Unniraman et al., 2004). However, a number of laboratories have shown that deregulated AID expression is not sufficient to induce B cell lymphoma in vivo (Muto et al., 2006; Okazaki et al., 2003; Shen et al., 2008). Our data resolve this apparent disparity and reveal that AID is indeed a cancer susceptibility gene that causes a broad spectrum of B cell malignancies. In contrast to gatekeeper genes that control cell proliferation and cell death or caretakers that regulate DNA repair, AID is a tumor promoter that destabilizes the genome by inducing DNA breaks and chromosome translocations.

AID creates DNA lesions by cytosine deamination (Petersen-Mahrt et al., 2002). Like other DSBs, AID breaks are recognized by proteins that mediate the DNA damage response, including H2AX, Nbs1, and 53BP1 (Manis et al., 2004; Petersen et al., 2001; Reina-San-Martin et al., 2005; Ward et al., 2004), and ATM (Difilippantonio et al., 2005; Reina-San-Martin et al., 2004), which, in turn, phosphorylates and recruits a canonical cascade of additional factors to the breaks (Jankovic et al., 2007). Although AID's activity is focused on antibody genes, where it produces high rates of mutation, its specificity is not absolute and its mechanism of targeting is still to be defined. The lack of absolute specificity results in low-frequency off-target mutation of oncogenes such as *Bcl6* and *c-myc* (Pasqualucci et al., 1998; Shen et al., 1998). However, the rate of AID-induced mutations in genes like *c-myc* is reduced by error-free repair by a yet to be determined UNG-dependent pathway,

resulting in mutation rates that are difficult to detect (Liu et al., 2008). Therefore, the complete list of genes that can be mutated by AID is not known.

Even less is understood about the extent to which the genome might be susceptible to AID-induced DSBs. Our experiments demonstrate that, although deregulating AID expression is not sufficient to overcome its targeting specificity, it induces a wide spectrum of chromosome aberrations, including reciprocal and nonreciprocal translocations and dicentric chromosomes, as well as chromosome deletions in primary B cells.

AID deregulation leads to increased somatic mutation and increases genomic instability but is not sufficient to cause malignant transformation. Conversely, loss of p53 was required for malignancy, but it did not significantly increase the level of translocation or DSBs. Thus, the presence of p53 did not prevent translocation per se, but p53 was required for transformation of cells bearing translocations. This would be in keeping with p53's ability to respond to oncogenic stress and induce the death of cells that overexpress oncogenes (Green and Kroemer, 2009; Hoffman and Liebermann, 2008; Kruse and Gu, 2009; Lowe et al., 2004). For example, p53-dependent cell death is triggered when *c-myc* is deregulated by the *Ig* enhancer in the *c-myc/IgH* translocation or by the *miR-142* promoter in the *c-myc/miR-142* translocation. Of note, p53 or its regulators are frequently mutated in human B cell malignancies, including diffuse large-B cell lymphoma, Burkitt's lymphoma, and chronic lymphocytic leukemia (reviewed in Kuppers, 2005). Finally, finding that there was no difference in the number of DSBs or chromosome abnormalities in the presence or absence of p53 indicates that the majority of the damage created by deregulated AID involves genes that do not activate p53. Based on our observations in mice, we speculate that p53's role in human lymphomas is to eliminate cells bearing AID-induced translocations that activate oncogenes.

Human B cell lymphomas can carry reciprocal translocations between two non-*Ig* genes; however, whether AID produces the lesions that lead to the malignancies associated with this class of translocations was not known. We found one example of a translocation in which neither of the two aberrantly joined non-*Ig* genes was an AID target as measured by somatic hypermutation (*Anxa4/Wdfy3*). However, the remaining non-*Ig* translocations in the B cell malignancies that arose in IgκAID/p53^{-/-} mice (n = 14) were associated with *c-myc/miR-142* translocation, which is also found in human B cell leukemia (Gauwerky et al., 1989). Both of these genes are AID targets. Consistent with bilateral initiation of these non-*Ig* translocations by AID,

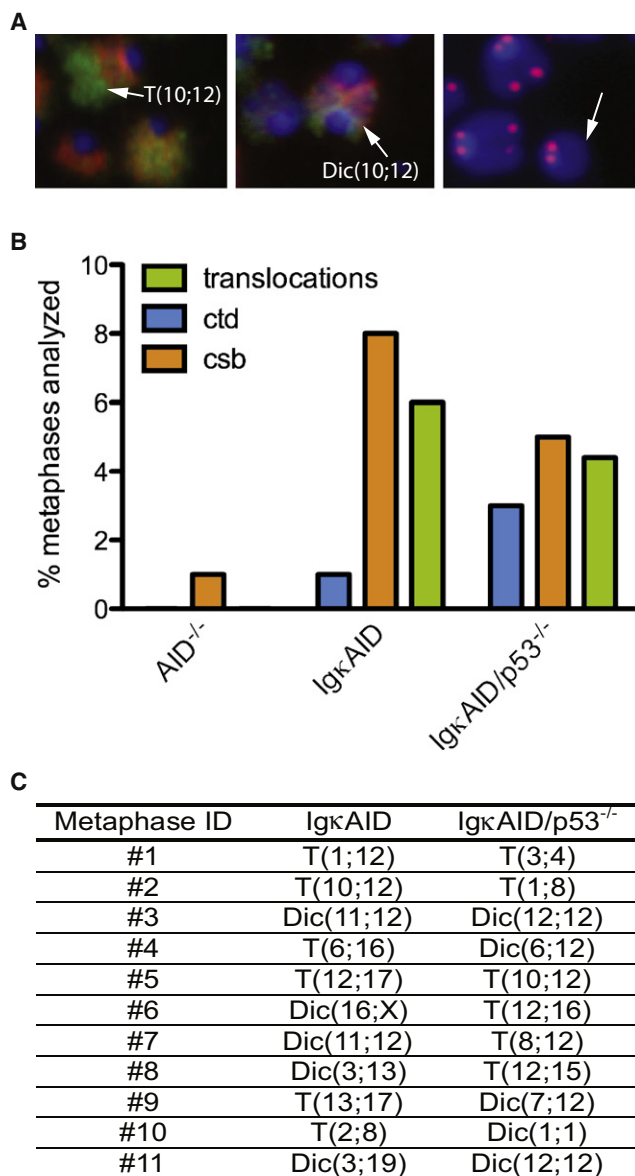


Figure 6. AID-Dependent Chromosome Instability in Stimulated Primary B Cells

(A) Representative M-FISH and telomere FISH images of B cells activated in vitro for 4 days with LPS, IL-4, and RP105. The arrows indicate (left to right) a translocation, a dicentric chromosome, and a chromosome break.

(B) Genomic instability and translocations in IgκAID primary B cells. Frequencies of ctd (chromatid breaks) and csb (chromosome breaks) were determined by analysis of metaphases hybridized with a telomere probe and counterstained with DAPI ($n = 100$). Frequency of translocations (including dicentrics) was determined by analysis of M-FISH images ($n \geq 250$).

(C) Table of chromosome aberrations detected in IgκAID and IgκAID/p53^{-/-} primary B cells. See also Table S3.

most showed junctional duplications that can only arise when the initiating lesions are staggered nicks on two DNA strands, including the *Anxa4/Wdfy3* translocation. Therefore, in addition to translocations involving *Ig*, AID can also induce cancer-associated translocations between two non-*Ig* genes. It is important

to note that somatic mutation, as currently measured, is not a sensitive indicator of whether or not a gene is an AID target simply because the rates of mutation sometimes approach the error rates of the polymerases used for PCR amplification. Thus, the lesions that lead to *Anxa4/Wdfy3* translocation, which show junctional duplication but no evidence of somatic mutation may still be due to AID. Given its destabilizing effects on the genome and oncogenic potential in vivo, it is not surprising that the expression of this enzyme and its residence in the nucleus are tightly regulated by a combination of cytoplasmic retention signals (Patenaude et al., 2009), nuclear export (McBride et al., 2004), phosphorylation (Cheng et al., 2009; McBride et al., 2006, 2008), micro-RNAs (Dorsett et al., 2008; Teng et al., 2008), and ubiquitylation (Aoufouchi et al., 2008).

In conclusion, AID is a potent endogenous tumor promoter, as it mutates oncogenes like *Bcl6* and creates DSBs in *Ig* and non-*Ig* genes that can serve as substrates for chromosome translocation. These lesions may account for a significant fraction of the mature B cell lymphomas that frequently arise in humans.

EXPERIMENTAL PROCEDURES

Mice

The IgκAID transgene is based on VkMYC (Robbiani et al., 2005) and includes the coding sequence of mouse AID (split over two exons) embedded in regulatory elements of the κ light-chain gene. The plasmid backbone was removed with MluI and NotI prior to pronuclear injection into C57BL/6 × (C57BL/6 × CBA) oocytes. Transgenic males were backcrossed to C57BL/6 for at least ten generations. AID^{-/-}, B1-8^{hi}, and p53^{-/-} mice were backcrossed to C57BL/6 (Casellas et al., 2001; Jacks et al., 1994; Muramatsu et al., 2000). UNG^{-/-} mice were previously described (Endres et al., 2004). All experiments were performed in accordance with protocols approved by the Rockefeller University and National Institutes of Health (NIH) Institutional Animal Care and Use Committee.

B Cell Cultures

Resting B lymphocytes were isolated from mouse spleens by immunomagnetic depletion with anti-CD43 MicroBeads (Miltenyi Biotec) and cultured at 0.5×10^6 cells/ml in R10 medium as previously described (Robbiani et al., 2008). For class switch recombination to IgG3, cells were stimulated with LPS (25 μg/ml, Sigma) and RP105 (0.5 μg/ml, PharMingen). For class switch recombination to IgG1, IL-4 (5 ng/ml, Sigma) was supplemented in addition to LPS and RP105. Cells were analyzed after 3 days of culture.

Flow Cytometry and Cell Sorting

For flow cytometric analysis, single-cell suspensions were stained with the indicated fluorochrome-conjugated antibodies (all from PharMingen). CFSE labeling for cell division was at 37°C for 10 minutes in 5 μM carboxyfluorescein succinimidyl ester (CFSE, Molecular Probes). Data were acquired on a FACSCalibur or LSRII instrument (Becton Dickinson) and analyzed with FlowJo software. Lymphoid populations were sorted to > 95% purity with a FACS Vantage SE with DIVA option or FACS Aria instruments (Becton Dickinson). Follicular B cells (CD19⁺CD23⁺CD21⁺) from naive spleens and bone marrow pre-B cells (B220⁺CD43^{low/int}IgM⁻IgD⁻) were enriched with anti-CD19 microbeads (Miltenyi Biotec). Splenic germinal center B cells (CD19⁺GL7⁺CD95⁺) from *P. chabaudi*-infected mice (day 14) were enriched with anti-CD19 microbeads prior to sorting.

Germinal Center Induction

Plasmodium chabaudi chabaudi clone AS (MRA 429) was obtained through the MR4 (Peters and Robinson, 2000). Parasites were maintained as frozen stocks and passaged in mice as described previously (Meding et al., 1990). For

experiments, mice were injected intraperitoneally with 10^5 infected red blood cells. Infections were monitored by microscopic examination of Giemsa-stained thin blood smears.

Western Blot

AID expression was analyzed as previously described (McBride et al., 2008). Lymphoid cells were purified as described above from *AID*^{-/-}, wild-type, or *IgkAID* mice. Activated splenic B cells were stimulated in vitro with LPS and IL-4 for 4 days.

PCR Assay for Translocations

DNA was extracted from resting splenic B cells or B cells that were stimulated in vitro for 4 days with LPS and IL-4. PCR reactions were performed on genomic DNA from 10^5 cells using the Expand Long Template PCR System (Roche). Nested reactions were performed as detailed and confirmed by Southern blot as described (Robbiani et al., 2008).

Mutational Analysis

The following primers were used to amplify the indicated genes with Pfu polymerase (Stratagene) and 25 cycles of amplification prior to cloning in TOPO vector (Invitrogen) and sequencing. *V_H*(B1-8^h): 5-CCATGGGATGGAGCTG TATCATCC-3 and 5-GAGGAGACTGTGAGAGTGGTCC-3. *S_μ*: 5-GACCCA GGCTAAGAAGGCAATC-3 and 5-GCGGCCCGGCTCATTCCAGTTCATTAC AG-3. *c-myc*: 5-TGGTCTTTCCCTGTGTTCTTTCTG-3 and 5-GACACCTCCC TTCTACACTCTAAACCG-3. *Taci*: 5-GTCAGGTCAGACAACCTCAGGAAGG-3 and 5-GTTTGCCACCCACATCAAGC-3. *Whsc1*: 5-ACGACGAAACGGTATGA AATCG-3 and 5-AAAAATGAAGGCTGCTGGGC-3. *H2Eα*: 5-CCAGAGACCAG GATGCCGC-3 and 5-TGGGCACCTTAGCACCGTAGTTAC-3. A20: 5-GGAC CATGGCTGAACAACCTT-3 and 5-ATCTGGCCGTTTGAGACAAC-3. *miR-142*: 5-CGTTGGATTCAAGACTGTGGGTC-3 and 5-AATGAGGGCGTGTGAGAGAT GCTC-3. *Anxa4*: 5-ACGGCACCATCTTCTGCTGTC-3 and 5-TCCTCCACACC TTGTTCTCTTGAG-3. *Wdfy3*: 5-AGAGGAGCCTGGTTTATGTAGCAG-3 and 5-TGGGAGGCTTATTGATTAGGCTG-3. Activated B cells were stimulated for 4 days with LPS and IL-4. Single B cell preparations from matched mice were compared. For additional details, see Table S4. P values were determined with the Student's t test using a two-tailed distribution.

Southern Blot

Rearrangements at the *c-myc* and *miR-142* loci were determined by digesting DNA with SspI (*c-myc*) and PstI (*miR-142*) and probing with PCR products of 5-CATTCTGACTCCTTTTGCCC-3 and 5-TCAGAGGTGGCTATTCAAGTTGC-3 (*c-myc*) or 5-CCCAGGCATTTTTCCACG-3 and 5-TTGAATCCAACGGAGG CAGC-3 (*miR-142*).

qPCR

Reverse-transcribed RNA from tumors and controls were amplified for *c-myc* (5-GCCCTAGTGCTGCATGAG-3 and 5-CCACAGACACCACATCAATTTT CTT-3) and normalized with *Gapdh* (5-TGAAGCAGGACTCTGAGG-3 and 5-CG AAGGTGAAGAGTGGGAG-3) using SYBR Green (Applied Biosystems).

RT-PCR and PCR for T(11;15) Breakpoints

Der11 *c-myc/miR-142* fusion transcripts were amplified with 5-AGTCGGCAA GAAAAGCAGGTG-3 and 5-TCGTCGCAGATGAAATAGGGC-3 and verified by direct sequencing. Der11 breakpoints were amplified with 5-TCGCTCTGC TGTGCTGGTGATAG-3 and 5-TTGTCGCTGGTTTCTGTGAG-3. Der15 breakpoints were amplified with 5-GTGAAACCGACTGTGGCCCTGGAA-3 and 5-CACAACCCCAATAACAGAGTCAGAC-3. The breakpoint was determined by direct sequencing of the gel-extracted PCR products.

Array Painting

Tumor cells growing in culture were arrested by overnight treatment with colcemid. The cells were treated with hypotonic KCl solution and 0.25% TX-100 before incubation with Chromomycin A3 (final concentration 40 pg/ml) and Hoechst 33258 (final concentration 2 pg/ml). Sorting was performed on a FACStar Plus flow sorter (Becton Dickinson) equipped with two argon ion lasers. Five thousand chromosomes were sorted. Amplification of sorted chromosomes was achieved using WGA4 (Sigma). Oligonucleotide array

CGH (aCGH) using mouse 244K tiling arrays was performed according to the manufacturer's protocol (Agilent Oligonucleotide Array-Based CGH for Genomic DNA Analysis, version 4.0, June 2006). Unsorted liver genomic DNA from sex-matched littermates was used as reference DNA. Slides were scanned using a G2565BA scanner, version 9.1. Feature Extraction software was applied, and the data were visualized using CGH Analytics 3.4.40 (Agilent).

M-FISH and Analysis of Genomic Instability

For analysis of chromosome and chromatid breaks, metaphase spreads were prepared from tumor cells and hybridized with telomere repeat-specific peptide nucleic acid (PNA) labeled with Cy3 (Applied Biosystems; Callén et al., 2007). Metaphases were counterstained with DAPI. For M-FISH, metaphases were hybridized with the 21× mouse probe cocktail (Metasystems). FISH-labeled metaphases were imaged using a Zeiss Axiomager M1, equipped with a motorized scanning stage, and analyzed using Isis software.

SUPPLEMENTAL DATA

Supplemental Data include nine figures and four tables and can be found with this article online at [http://www.cell.com/molecular-cell/supplemental/S1097-2765\(09\)00820-X](http://www.cell.com/molecular-cell/supplemental/S1097-2765(09)00820-X).

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REFERENCES

- Aoufouchi, S., Faili, A., Zober, C., D'Orlando, O., Weller, S., Weill, J.C., and Reynaud, C.A. (2008). Proteasomal degradation restricts the nuclear lifespan of AID. *J. Exp. Med.* 205, 1357–1368.
- Bassing, C.H., Suh, H., Ferguson, D.O., Chua, K.F., Manis, J., Eckersdorff, M., Gleason, M., Bronson, R., Lee, C., and Alt, F.W. (2003). Histone H2AX: a dosage-dependent suppressor of oncogenic translocations and tumors. *Cell* 114, 359–370.
- Bassing, C.H., Swat, W., and Alt, F.W. (2002). The mechanism and regulation of chromosomal V(D)J recombination. *Cell* 109 (Suppl.), S45–S55.
- Callén, E., Jankovic, M., Difilippantonio, S., Daniel, J.A., Chen, H.T., Celeste, A., Pellegrini, M., McBride, K., Wangsa, D., Bredemeyer, A.L., et al. (2007). ATM prevents the persistence and propagation of chromosome breaks in lymphocytes. *Cell* 130, 63–75.
- Callén, E., Bunting, S., Huang, C.Y., Difilippantonio, M.J., Wong, N., Khor, B., Mahowald, G., Kruhlak, M.J., Ried, T., Sleckman, B.P., et al. (2009). Chimeric IgH-TCR/alpha/delta translocations in T lymphocytes mediated by RAG. *Cell Cycle* 8, 2408–2412.
- Casellas, R., Shih, T.A., Kleinewietfeld, M., Rakonjac, J., Nemazee, D., Rajewsky, K., and Nussenzweig, M.C. (2001). Contribution of receptor editing to the antibody repertoire. *Science* 291, 1541–1544.
- Chen, C.Z., Li, L., Lodish, H.F., and Bartel, D.P. (2004). MicroRNAs modulate hematopoietic lineage differentiation. *Science* 303, 83–86.

- Cheng, H.L., Vuong, B.Q., Basu, U., Franklin, A., Schwer, B., Astarita, J., Phan, R.T., Datta, A., Manis, J., Alt, F.W., et al. (2009). Integrity of the AID serine-38 phosphorylation site is critical for class switch recombination and somatic hypermutation in mice. *Proc. Natl. Acad. Sci. USA* 106, 2717–2722.
- Difilippantonio, S., Celeste, A., Fernandez-Capetillo, O., Chen, H.T., Reina San Martin, B., Van Laethem, F., Yang, Y.P., Petukhova, G.V., Eckhaus, M., Feigenbaum, L., et al. (2005). Role of Nbs1 in the activation of the Atm kinase revealed in humanized mouse models. *Nat. Cell Biol.* 7, 675–685.
- Di Noia, J.M., and Neuberger, M.S. (2007). Molecular mechanisms of antibody somatic hypermutation. *Annu. Rev. Biochem.* 76, 1–22.
- Dorsett, Y., Robbiani, D.F., Jankovic, M., Reina-San-Martin, B., Eisenreich, T.R., and Nussenzweig, M.C. (2007). A role for AID in chromosome translocations between c-myc and the IgH variable region. *J. Exp. Med.* 204, 2225–2232.
- Dorsett, Y., McBride, K.M., Jankovic, M., Gazumyan, A., Thai, T.H., Robbiani, D.F., Di Virgilio, M., San-Martin, B.R., Heidkamp, G., Schwickert, T.A., et al. (2008). MicroRNA-155 suppresses activation-induced cytidine deaminase-mediated Myc-Igh translocation. *Immunity* 28, 630–638.
- Dudley, D.D., Chaudhuri, J., Bassing, C.H., and Alt, F.W. (2005). Mechanism and control of V(D)J recombination versus class switch recombination: similarities and differences. *Adv. Immunol.* 86, 43–112.
- Endres, M., Biniszkiwicz, D., Sobol, R.W., Harms, C., Ahmadi, M., Lipski, A., Katchanov, J., Mergenthaler, P., Dirnagl, U., Wilson, S.H., et al. (2004). Increased postischemic brain injury in mice deficient in uracil-DNA glycosylase. *J. Clin. Invest.* 113, 1711–1721.
- Gauwerky, C.E., Huebner, K., Isobe, M., Nowell, P.C., and Croce, C.M. (1989). Activation of MYC in a masked t(8;17) translocation results in an aggressive B-cell leukemia. *Proc. Natl. Acad. Sci. USA* 86, 8867–8871.
- Greaves, M.F., and Wiemels, J. (2003). Origins of chromosome translocations in childhood leukaemia. *Nat. Rev. Cancer* 3, 639–649.
- Green, D.R., and Kroemer, G. (2009). Cytoplasmic functions of the tumour suppressor p53. *Nature* 458, 1127–1130.
- Gribble, S.M., Kalaitzopoulos, D., Burford, D.C., Prigmore, E., Selzer, R.R., Ng, B.L., Matthews, N.S., Porter, K.M., Curley, R., Lindsay, S.J., et al. (2007). Ultra-high resolution array painting facilitates breakpoint sequencing. *J. Med. Genet.* 44, 51–58.
- Hoffman, B., and Liebermann, D.A. (2008). Apoptotic signaling by c-MYC. *Oncogene* 27, 6462–6472.
- Honjo, T., Kinoshita, K., and Muramatsu, M. (2002). Molecular mechanism of class switch recombination: linkage with somatic hypermutation. *Annu. Rev. Immunol.* 20, 165–196.
- Inagaki, H. (2007). Mucosa-associated lymphoid tissue lymphoma: molecular pathogenesis and clinicopathological significance. *Pathol. Int.* 57, 474–484.
- Jacks, T., Remington, L., Williams, B.O., Schmitt, E.M., Halachmi, S., Bronson, R.T., and Weinberg, R.A. (1994). Tumor spectrum analysis in p53-mutant mice. *Curr. Biol.* 4, 1–7.
- Jankovic, M., Nussenzweig, A., and Nussenzweig, M.C. (2007). Antigen receptor diversification and chromosome translocations. *Nat. Immunol.* 8, 801–808.
- Kovalchuk, A.L., duBois, W., Mushinski, E., McNeil, N.E., Hirt, C., Qi, C.F., Li, Z., Janz, S., Honjo, T., Muramatsu, M., et al. (2007). AID-deficient Bcl-xL transgenic mice develop delayed atypical plasma cell tumors with unusual Ig/Myc chromosomal rearrangements. *J. Exp. Med.* 204, 2989–3001.
- Kruse, J.P., and Gu, W. (2009). Modes of p53 regulation. *Cell* 137, 609–622.
- Kuppers, R. (2005). Mechanisms of B-cell lymphoma pathogenesis. *Nat. Rev. Cancer* 5, 251–262.
- Kuppers, R., and Dalla-Favera, R. (2001). Mechanisms of chromosomal translocations in B cell lymphomas. *Oncogene* 20, 5580–5594.
- Landgraf, P., Rusu, M., Sheridan, R., Sewer, A., Iovino, N., Aravin, A., Pfeffer, S., Rice, A., Kamphorst, A.O., Landthaler, M., et al. (2007). A mammalian microRNA expression atlas based on small RNA library sequencing. *Cell* 129, 1401–1414.
- Liao, M.J., Zhang, X.X., Hill, R., Gao, J., Qumsiyeh, M.B., Nichols, W., and Van Dyke, T. (1998). No requirement for V(D)J recombination in p53-deficient thymic lymphoma. *Mol. Cell. Biol.* 18, 3495–3501.
- Lieber, M.R., Yu, K., and Raghavan, S.C. (2006). Roles of nonhomologous DNA end joining, V(D)J recombination, and class switch recombination in chromosomal translocations. *DNA Repair (Amst.)* 5, 1234–1245.
- Lieber, M.R., Raghavan, S.C., and Yu, K. (2008). Mechanistic aspects of lymphoid chromosomal translocations. *J. Natl. Cancer Inst. Monogr.* 2008, 8–11.
- Liu, M., Duke, J.L., Richter, D.J., Vinuesa, C.G., Goodnow, C.C., Kleinstein, S.H., and Schatz, D.G. (2008). Two levels of protection for the B cell genome during somatic hypermutation. *Nature* 451, 841–845.
- Lowe, S.W., Cepero, E., and Evan, G. (2004). Intrinsic tumour suppression. *Nature* 432, 307–315.
- Manis, J.P., Morales, J.C., Xia, Z., Kutok, J.L., Alt, F.W., and Carpenter, P.B. (2004). 53BP1 links DNA damage-response pathways to immunoglobulin heavy chain class-switch recombination. *Nat. Immunol.* 5, 481–487.
- McBride, K.M., Barreto, V., Ramiro, A.R., Stavropoulos, P., and Nussenzweig, M.C. (2004). Somatic hypermutation is limited by CRM1-dependent nuclear export of activation-induced deaminase. *J. Exp. Med.* 199, 1235–1244.
- McBride, K.M., Gazumyan, A., Woo, E.M., Barreto, V.M., Robbiani, D.F., Chait, B.T., and Nussenzweig, M.C. (2006). Regulation of hypermutation by activation-induced cytidine deaminase phosphorylation. *Proc. Natl. Acad. Sci. USA* 103, 8798–8803.
- McBride, K.M., Gazumyan, A., Woo, E.M., Schwickert, T.A., Chait, B.T., and Nussenzweig, M.C. (2008). Regulation of class switch recombination and somatic mutation by AID phosphorylation. *J. Exp. Med.* 205, 2585–2594.
- McKean, D., Huppi, K., Bell, M., Staudt, L., Gerhard, W., and Weigert, M. (1984). Generation of antibody diversity in the immune response of BALB/c mice to influenza virus hemagglutinin. *Proc. Natl. Acad. Sci. USA* 81, 3180–3184.
- Meding, S.J., Cheng, S.C., Simon-Haerhaus, B., and Langhorne, J. (1990). Role of gamma interferon during infection with *Plasmodium chabaudi* chabaudi. *Infect. Immun.* 58, 3671–3678.
- Muramatsu, M., Kinoshita, K., Fagarasan, S., Yamada, S., Shinkai, Y., and Honjo, T. (2000). Class switch recombination and hypermutation require activation-induced cytidine deaminase (AID), a potential RNA editing enzyme. *Cell* 102, 553–563.
- Muto, T., Okazaki, I.M., Yamada, S., Tanaka, Y., Kinoshita, K., Muramatsu, M., Nagaoka, H., and Honjo, T. (2006). Negative regulation of activation-induced cytidine deaminase in B cells. *Proc. Natl. Acad. Sci. USA* 103, 2752–2757.
- Okazaki, I.M., Hiai, H., Kakazu, N., Yamada, S., Muramatsu, M., Kinoshita, K., and Honjo, T. (2003). Constitutive expression of AID leads to tumorigenesis. *J. Exp. Med.* 197, 1173–1181.
- Pasqualucci, L., Bhagat, G., Jankovic, M., Compagno, M., Smith, P., Muramatsu, M., Honjo, T., Morse, H.C., III, Nussenzweig, M.C., and Dalla-Favera, R. (2008). AID is required for germinal center-derived lymphomagenesis. *Nat. Genet.* 40, 108–112.
- Pasqualucci, L., Migliazza, A., Fracchiolla, N., William, C., Neri, A., Baldini, L., Chaganti, R.S., Klein, U., Kuppers, R., Rajewsky, K., et al. (1998). BCL-6 mutations in normal germinal center B cells: evidence of somatic hypermutation acting outside Ig loci. *Proc. Natl. Acad. Sci. USA* 95, 11816–11821.
- Patenaude, A.M., Orthwein, A., Hu, Y., Campo, V.A., Kavli, B., Buschiazzo, A., and Di Noia, J.M. (2009). Active nuclear import and cytoplasmic retention of activation-induced deaminase. *Nat. Struct. Mol. Biol.* 16, 517–527.
- Peled, J.U., Kuang, F.L., Iglesias-Ussel, M.D., Roa, S., Kalis, S.L., Goodman, M.F., and Scharff, M.D. (2008). The biochemistry of somatic hypermutation. *Annu. Rev. Immunol.* 26, 481–511.
- Peters, W., and Robinson, B.L. (2000). The chemotherapy of rodent malaria. LVIII. Drug combinations to impede the selection of drug resistance. Part 2: The new generation-artemisinin or artesunate with long-acting blood schizontocides. *Ann. Trop. Med. Parasitol.* 94, 23–35.

- Petersen, S., Casellas, R., Reina-San-Martin, B., Chen, H.T., Difilippantonio, M.J., Wilson, P.C., Hanitsch, L., Celeste, A., Muramatsu, M., Pilch, D.R., et al. (2001). AID is required to initiate Nbs1/gamma-H2AX focus formation and mutations at sites of class switching. *Nature* 414, 660–665.
- Petersen-Mahrt, S.K., Harris, R.S., and Neuberger, M.S. (2002). AID mutates *E. coli* suggesting a DNA deamination mechanism for antibody diversification. *Nature* 418, 99–103.
- Phan, R.T., and Dalla-Favera, R. (2004). The BCL6 proto-oncogene suppresses p53 expression in germinal-centre B cells. *Nature* 432, 635–639.
- Potter, M. (2003). Neoplastic development in plasma cells. *Immunol. Rev.* 194, 177–195.
- Rabbitts, T.H. (2009). Commonality but diversity in cancer gene fusions. *Cell* 137, 391–395.
- Ramiro, A.R., Jankovic, M., Eisenreich, T., Difilippantonio, S., Chen-Kiang, S., Muramatsu, M., Honjo, T., Nussenzweig, A., and Nussenzweig, M.C. (2004). AID is required for c-myc/IgH chromosome translocations in vivo. *Cell* 118, 431–438.
- Ramiro, A.R., Jankovic, M., Callen, E., Difilippantonio, S., Chen, H.T., McBride, K.M., Eisenreich, T.R., Chen, J., Dickins, R.A., Lowe, S.W., et al. (2006a). Role of genomic instability and p53 in AID-induced c-myc-IgH translocations. *Nature* 440, 105–109.
- Ramiro, A.R., Nussenzweig, M.C., and Nussenzweig, A. (2006b). Switching on chromosomal translocations. *Cancer Res.* 66, 7837–7839.
- Ramiro, A., San-Martin, B.R., McBride, K., Jankovic, M., Barreto, V., Nussenzweig, A., and Nussenzweig, M.C. (2007). The role of activation-induced deaminase in antibody diversification and chromosome translocations. *Adv. Immunol.* 94, 75–107.
- Reina-San-Martin, B., Chen, H.T., Nussenzweig, A., and Nussenzweig, M.C. (2004). ATM is required for efficient recombination between immunoglobulin switch regions. *J. Exp. Med.* 200, 1103–1110.
- Reina-San-Martin, B., Nussenzweig, M.C., Nussenzweig, A., and Difilippantonio, S. (2005). Genomic instability, endoreduplication, and diminished Ig class-switch recombination in B cells lacking Nbs1. *Proc. Natl. Acad. Sci. USA* 102, 1590–1595.
- Robbiani, D.F., Colon, K., Affer, M., Chesi, M., and Bergsagel, P.L. (2005). Maintained rules of development in a mouse B-cell tumor. *Leukemia* 19, 1278–1280.
- Robbiani, D.F., Bothmer, A., Callen, E., Reina-San-Martin, B., Dorsett, Y., Difilippantonio, S., Bolland, D.J., Chen, H.T., Corcoran, A.E., Nussenzweig, A., et al. (2008). AID is required for the chromosomal breaks in c-myc that lead to c-myc/IgH translocations. *Cell* 135, 1028–1038.
- Schatz, D.G. (2004). V(D)J recombination. *Immunol. Rev.* 200, 5–11.
- Schlissel, M.S., Kaffer, C.R., and Curry, J.D. (2006). Leukemia and lymphoma: a cost of doing business for adaptive immunity. *Genes Dev.* 20, 1539–1544.
- Shaffer, A.L., Rosenwald, A., and Staudt, L.M. (2002). Lymphoid malignancies: the dark side of B-cell differentiation. *Nat. Rev. Immunol.* 2, 920–932.
- Shen, H.M., Peters, A., Baron, B., Zhu, X., and Storb, U. (1998). Mutation of BCL-6 gene in normal B cells by the process of somatic hypermutation of Ig genes. *Science* 280, 1750–1752.
- Shen, H.M., Bozek, G., Pinkert, C.A., McBride, K., Wang, L., Kenter, A., and Storb, U. (2008). Expression of AID transgene is regulated in activated B cells but not in resting B cells and kidney. *Mol. Immunol.* 45, 1883–1892.
- Stavnezer, J., Guikema, J.E., and Schrader, C.E. (2008). Mechanism and regulation of class switch recombination. *Annu. Rev. Immunol.* 26, 261–292.
- Takizawa, M., Tolarova, H., Li, Z., Dubois, W., Lim, S., Callen, E., Franco, S., Mosaico, M., Feigenbaum, L., Alt, F.W., et al. (2008). AID expression levels determine the extent of cMyc oncogenic translocations and the incidence of B cell tumor development. *J. Exp. Med.* 205, 1949–1957.
- Teng, G., and Papavasiliou, F.N. (2007). Immunoglobulin somatic hypermutation. *Annu. Rev. Genet.* 41, 107–120.
- Teng, G., Hakimpour, P., Landgraf, P., Rice, A., Tuschl, T., Casellas, R., and Papavasiliou, F.N. (2008). MicroRNA-155 is a negative regulator of activation-induced cytidine deaminase. *Immunity* 28, 621–629.
- Tonegawa, S. (1983). Somatic generation of antibody diversity. *Nature* 302, 575–581.
- Tsai, A.G., Lu, H., Raghavan, S.C., Muschen, M., Hsieh, C.L., and Lieber, M.R. (2008). Human chromosomal translocations at CpG sites and a theoretical basis for their lineage and stage specificity. *Cell* 135, 1130–1142.
- Unniraman, S., Zhou, S., and Schatz, D.G. (2004). Identification of an AID-independent pathway for chromosomal translocations between the IgH switch region and Myc. *Nat. Immunol.* 5, 1117–1123.
- Wang, J.H., Gostissa, M., Yan, C.T., Goff, P., Hickernell, T., Hansen, E., Difilippantonio, S., Wesemann, D.R., Zarrin, A.A., Rajewsky, K., et al. (2009). Mechanisms promoting translocations in editing and switching peripheral B cells. *Nature* 460, 231–236.
- Ward, I.M., Reina-San-Martin, B., Olaru, A., Minn, K., Tamada, K., Lau, J.S., Cascalho, M., Chen, L., Nussenzweig, A., Livak, F., et al. (2004). 53BP1 is required for class switch recombination. *J. Cell Biol.* 165, 459–464.